THE SPECTROPHOTOMETRIC CHARACTERISTICS OF THE HEMOGLOBIN OF FOWLS

by

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THE SPECTROPHOTOMETRIC CHARACTERISTICS OF THE HEMOGLOBIN OF FOWLS

INTRODUCTION

It has been observed in the routine determinations of hemoglobin in chicken blood by members of this Experiment Station that the acid hematin derivative does not match satisfactorily colorimetric standards prepared from the blood of mammals. Standards prepared from chicken blood soon become cloudy and unusable. The hemoglobin itself does not satisfactorily match the tint of the Fleischl-Miescher hemometer standard used for the blood of higher animals. The Van Slyke method encounters several difficulties because of certain peculiar characteristics of chicken blood, and is at any rate too cumbersome for a routine method.

The purpose of this work is to study the spectrum absorption of the hemoglobin of fowls, particularly chickens, and to determine the absorption coefficient by means of which direct quantitative determinations of chicken hemoglobin can be made in the spectrophotometer without the use of any prepared or artificial standard.

When a beam of light is passed through a substance there is for every homogeneous colored solution a characteristic absorption of definite spectrum regions. This absorbed light may be transformed into heat or into fluorescent or phosphorescent light of wavelengths different from its own, or it may cause ionization or chemical reaction. The rest of the light lost is either scattered at the surface or in the interior and some is irregularly reflected at the surface. But since only the

absorbed light is significant to the chemist, allowance must be made for the other lost light, in taking measurements. This can be compensated for by arranging that another non-absorbing substance with reflecting surfaces similar to that to be measured is used for comparison.

That the intensity of the source of light bears no relation to the amount absorbed by the solution has been shown by Lambert (1). He has shown, however, that there is a definite relationship between the concentration of the solution and the intensity of the transmitted light or the amount of light absorbed. He has made use of the following equations, which later investigators have accepted and used:

> If I_0 is the intensity of the incident light beam and I is the intensity of the emergent beam through a path length d, and 1/a is the path length, passage through which reduces the light intensity 1/10th, and is constant depending on the medium, and is called the extinction coefficient, then $I = I_0 \cdot 10^{-da}$.

Twyman (2) and other workers have investigated Beer's law and found it correct. It states that if an absorbing salt is dissolved in a non-absorbing liquid, its absorption of a beam of homogeneous light depends on the number of molecules of the absorbing substance which the beam of light passes through, that is the concentration of the solution.

> If I, I_0, and d, and a, have the same significance as above, $I = I_0 a^{-dc}$ or if a_0 is the extinction coefficient of a solution

for unit thickness and concentration, then

$I = I_0 10^{-da_0 c}$

where c is the concentration.

Another term which is often useful is the density, D.

$D = -da_0 c$

$I = I_0 10^{-D}$

and $D = \log_{10} \frac{I_0}{I}$

$\log_{10} \frac{I}{I_0} = \log T$

where T is the transmission

therefore $\log_{10} \frac{I_0}{I} = -\log T$

and $D = -\log T$.

REVIEW OF LITERATURE

Quantitative hemoglobin determinations of the blood of mammals have been of importance for many years in the study of nutrition, pathology and medicine.

Any very accurate method of analysis depends on the preparation of the pure hemoglobin crystals for a fundamental basis of comparison; but this method besides being long and tedious requires a great quantity of blood which is not always easily obtained from one source. Another well-known method which is fairly recent, is that of Van Slyke (3). It is based upon the principle that the hemoglobin molecule absorbs a definite amount of oxygen in the formation of oxyhemoglobin, which can be easily released when treated with certain agents and placed under vacuum.

The amount of hemoglobin present in a sample can be calculated if the volume of oxygen released has been measured. Although accurate, this method is too cumbersome for a routine method.

Colorimetric methods are widely used because they can be quickly and easily performed. These methods are measured by a psychological or color sensation and due to this fact are subject to abnormalities of the observers' color vision.

Perhaps the most accurate method of hemoglobin analysis, also based upon color determination and specification, is the spectrophotometric method. Color is analyzed by measurement of the actual radiant energy producing the sensation. Therefore in measuring the actual stimulus it removes the factor of differences in tint and reduces the measurement to a comparison of light intensities. The intensity of the transmitted light is taken at the point on the spectrum at which maximum absorption occurs, while ordinary colorimetric methods involve the inspection of the entire light transmitted. Since the spectrophotometric method is both rapid and accurate, it has come into common use in the modern research laboratory.

The quantitative relationship between the absorption of light and the concentration of hemoglobin solutions have been subjected to experimental tests by many investigators ever since K. Vierordt (1873) pointed out the value of spectrophotometry in biological work. The factor which expresses this qualitative relationship is designated as the absorption ratio (a) which is a constant for a particular substance and at a given

wave length. The determination of the value of this constant for hemoglobin depends not only upon a spectrophotometric method of measurement but also upon some other method which determines the concentration of the solution used. Data accumulated from early workers are based upon spectrophotometric absorption measurements made upon solutions of weighed amounts of crystallized oxyhemoglobin in a definite volume of water.

The absorption spectrum of oxyhemoglobin exhibits two maxima, one in yellow and one in the green. The absorption measurements are made at a point in the spectrum where maximum absorption occurs in order that the greatest amount of variation in light absorption may occur per unit concentration. The measurements are usually made on the maximum in the green because of the greater ease with which the eye accommodates itself to the color.

There has been some difference of opinion concerning the point on the spectrum at which maximum absorption occurs in the green band and the absorption factor relating concentration to optical density. Data accumulated from early workers is shown in Table I, below.

Table I

Absorption Factors Obtained by Early Workers

Investigator	Date	Maximum Point	Absorption Factor
Bordachsi	1906	542-551	1.33
Butterfield	1909	542-535	1.195
Litsche	1911	543-534	1.52
Hari	1917	541-533	1.168
Williamson	1916	542-534	1.179
Newcomer	1919	543-539	1.14
Kennedy	1926	540	1.165

In 1924 Van Slyke outlined an oxygen capacity method of hemoglobin determination which has proved satisfactory for a quantitative analysis of hemoglobin solutions. Optical instruments have been greatly improved since the early workers, so in 1926 Robert P. Kennedy (3) repeated the work of spectrophotometric studies using as a basis for optical constants an analysis of oxygen capacity. The oxygen capacity of hemoglobin had been established already and found to be constant.

Kennedy using as experimental animals--dogs, donkeys and man, followed strictly the method outlined by Van Slyke and from this data calculated the grams of hemoglobin per 100 c.c. of blood. The instrument he used for the spectrophotometric determinations was a Bausch and

Lomb instrument of a similar type used in supplying the data for this paper, and will be described later.

Kennedy obtained the following results by plotting the wave length against the absorption:

A maximum in the green at 540 millimu,

A maximum in the yellow at 575 millimu,

and a minimum between the two at 560 millimu.

The ratio $\frac{D540}{D560} = \frac{1.40}{.86} = 1.63$

The absorption ratio determined at the maximum in the green, with the aid of the concentration data obtained by the Van Slyke method, was 1.165 mg. hemoglobin per c.c. solution.

All of the above data have been obtained from the blood of higher animals. There is very little literature, however, concerning the spectrophotometric characteristics of fowl blood.

The only reference found in the literature was an article written by Dr. G. Fritsch of the University of Giessen and performed by K. Bürker and co-workers of the same university. They obtained an absorption ratio of 1.25, determined at a maximum of 555.6-542.1 millimu. These results are questionable since no accurate results could be expected using a maximum over a range of 6.5 mM. Upon further investigation, it was found that in an earlier article Bürker (5) obtained the same factor 1.25 for the blood of higher animals. Since this does not agree with either the work of early investigators, or with the widely accepted re-

sults obtained by Robert Kennedy. It is highly probable that Barker's results should be regarded as questionable.

METHODS

Van Slyke Determinations

The sample of venous blood taken from the wing vein of the fowl by means of a hypodermic needle was citrated to prevent coagulation, then placed in a large mouthed flask and slowly rotated for fifteen minutes so that complete aeration was obtained. The aerated blood was transferred to a small weighing bottle until ready for use. The latter is a precaution to obtain thorough mixing, as the blood can be thoroughly stirred by the pipette while the sample is being taken. It was found that the large cells of chicken blood tend to settle out from the plasma very rapidly. Samples for the spectrophotometric study were taken at the same time as samples for the Van Slyke determinations, so as to avoid possibilities of loss or change by evaporation or decomposition.

The concentration determinations as before stated were made by use of the well-known Van Slyke method. The blood sample is introduced into the apparatus under a solution of potassium ferricyanide and saponin. The mixture is put under vacuum and shaken for three minutes. The combined effects of the saponin-potassium ferricyanide reagent and the vacuum free the combined oxygen from the oxyhemoglobin. The partial pressure of the released oxygen (the CO_2 having been absorbed by a normal solution of sodium hydroxide) is measured by a mercury manometer and from this data the volume of gas at standard conditions or the volume

percent is calculated by use of a formula developed by Van Slyke (6). The volume of dissolved nitrogen gas also released during the above process is subtracted and the volume percent oxygen found. This quantity multiplied by the factor 0.746 gives the concentration in grams of hemoglobin per 100 c.c. of blood. Determinations were repeated until check results were obtained. The formula for calculating the total gas content as developed by Van Slyke:

$V = aP$ where V = volume percent gas

where a = volume percent factor

and P = partial pressure of gas

The term (a) is a constant for a certain apparatus at a certain temperature and can be found in tables arranged by Van Slyke (7).

$V \cdot .746$ = grams Hemoglobin per 100 c.c. blood

THE SPECTROPHOTOMETER

It has long been recognized that polarization photometry is one of the simplest and quickest methods for comparison of light intensities. The Bausch and Lomb spectrophotometer, the instrument here used, consists of three units: (1) A means of illumination for the sample, (2) a rapid means of varying and comparing light intensities, (3) a spectrometer which divides the transmitted beam into its constituent colored components. There are two vertical specimen holders between the photometer and the source of light. Right angle prisms reflect the light beams so that they pass vertically downward through the cups and plungers

The cups are movable, and a suitable lens system serves to give parallel light through the cups and plungers. The parallel beams are focused on the photometer apertures, giving an intense illumination in the field of the photometer. The two halves of the comparison field are polarized at right angles to each other by means of a Wollaston prism. A lens images the photometric field upon the slit of the spectrometer.

The spectrometer re-images the field and its dividing line in the focal plane of the telescope objective giving two adjacent spectra. A shutter eyepiece allows a rectangular section vertical to the dividing line and of any desired width to be selected and compared. The length of the field and the intensity of illumination are respectively controlled at the entrance slit of the spectrometer by the V slide and the micrometer screw. The two halves of the rectangular field thus formed are brought to equality of brightness by means of the analyzer of the photometer which is operated from the observer's position at the eyepoint of the spectrometer. Thus successive portions of the spectrum may be examined and compared and from the data a complete transmission or density curve plotted. The zero point may not be exactly 45° on account of the slight polarization at the surfaces of the bi-prism and mechanical adjustments, but compensation is made for any such small deviations by reversing the photometer for each reading.

THE SPECTROPHOTOMETRIC METHOD

The spectrophotometer was placed before a source of sodium light and adjusted so that the spectral lines were sharply in focus. The wave length drum was then turned to the graduation corresponding to sodium line 590 mM and the shutter adjusted until it centered evenly on the corresponding sodium line. Readings were taken on the principle mercury and lithium lines. The average derivation from the accepted values in the critical tables was .1 mM; at the extremities of the spectrum, however, there was a deviation of .5 mM.

The apparatus was further checked by a method as outlined by the Bureau of Standards (8). Amaranth dye was dissolved in an acetate buffer mixture placed in a cup of the spectrophotometer and the density determined at different wave lengths along the entire length of the visible spectrum. In plotting the optical density against the wave length, it was found that the maximum point of absorption occurred at 520.8 mM, which checked within .2 mM of that obtained by the Bureau of Standards.

Chicken blood samples previously analyzed for hemoglobin concentration, were first diluted 1-100 in a 0.1% Na_2CO_3 solution. The solution was then mixed with considerable agitation so that the blood was completely laked. After shaking a gelantinous material appeared in the solution, the volume of which was much greater than the volume of the blood before dilution. An attempt was made to remove the material by centrifuging as it was thought that it would probably be

thrown to the top and could then be easily removed from the solution. However, the results did not prove satisfactory, since the material still remained distributed throughout the solution. Then filtration was tried but enough hemoglobin remained occluded in the gelatinous material to give quantitatively inaccurate results. Next the blood was diluted 1-250 in a 0.1% Na_2CO_3 solution and after vigorous shaking it was found that the material was sufficiently dispersed so that it could be filtered from the solution without retaining any noticeable amount of hemoglobin.

The filterable residue was devoid of any red tint and the filtrate showed no Tyndall beam when placed in the photometer cups.

Therefore this method was used in preparing the blood dilutions. A cup was filled with the solution and the opposite cup with water and the plungers placed at a depth of 2.5 cm. This arrangement provides an equal optical absorption to a 1-100 dilution at the customary 1 cm. depth, and it places the readings on normal blood in the most accurate range of the photometer. The spectrometer was set at a certain wavelength and the analyzing prism of the photometer rotated until a match in the comparison field was secured. The photometer then was reversed and a match again secured. The wavelength drum then was changed and similar readings taken until the range of the visible spectrum was covered.

If the zero point of the instrument is exactly 45°, the formula given for transmission is: $\tan^2\theta_1 = T$; however, most instruments devi-

ate slightly in their match point from 45°. This is compensated by taking readings with the photometer first in one position and then in the other. If θ_1 is the large angle of rotation, and θ_2 is the small angle in the opposite position, then $T = \frac{\tan \theta_2}{\tan \theta_1} = \tan \theta_2 \text{ tot } \theta_1$.

To express absorption in terms that are directly proportional to concentration, at first all absorption measurements are expressed in terms of optical density. (See Table II.)

$$D = -\log T = \log \tan \theta_2 + \log \cot \theta_1$$

When the optical density was plotted against the wave length, maxima were obtained at 542 and 577 and a minimum between the two at 562. (See Figure I.) These critical points are identical with those found by Hunk (9) for beef blood hemaglobin, but are displaced two mμ toward the red from values obtained by Kennedy.

After establishing the maxima and the minimum points on the absorption diagram of fowl hemoglobin, the blood of a group of chickens varying in breed, age and sex was investigated at these three important points and the absorption factors determined at the point of maximum absorption in the green. Duplicate dilutions for spectrophotometric analysis were made on each sample at the time Van Slyke determinations were made. No difficulty whatever was encountered in obtaining checks on the optical density. More difficulty appeared in obtaining checks in the oxygen capacity. For this reason it seems likely that the spectrophotometric method is more accurate when once the absorption factor is established.

The ratios of the optical density at the maxima to the optical density at the minimum; and the maximum in the yellow to the maximum in the green were found with the results shown in table III. The ratio obtained for the absorptions at 542 mμ to 562 mμ was 1.63 as compared to 1.632 obtained by John Shenk in his thesis (9) on "A Spectrophotometric Analysis of Blood and Muscle Hemoglobin Solutions."

The ratio of the absorption at 577 mμ to 562 mμ was 1.69 as compared to 1.7 found by John Shenk. And the ratio 577 mμ to 562 mμ was 1.05 as compared to 1.054 obtained by John Shenk.

For comparison, a number of samples of beef blood were then analyzed and the absorption curve plotted. The curve was identical with that obtained from chicken blood. However, the absorption factors differed from those obtained in the chicken blood analysis. (See Table IV.) The beef hemoglobin factors remained constant at 1.185, agreeing with the absorption factor of mammalian blood obtained by Kennedy.

A group of sixteen turkeys, including both male and female, were investigated in the same manner and it was found that here too the absorption factor varied as in the chicken blood. (Table V.)

Finding that the absorption factor varied for the different chickens, the next step was to find whether the absorption factor obtained from the blood of the same chicken varied from day to day.

Six roosters of different breeds were bled at intervals of every other day or once a week until an average of eight determinations was made on each rooster. Then another group of six roosters was treated

in the same manner until with the combined results one hundred determinations were made on twelve roosters. The results as given in Table VI show that the absorption ratio of the hemoglobin of the same fowl varies from day to day.

In order to compare these results with the characteristics of mammalian blood, three calves were bled once every two weeks for six weeks and the blood analyzed in the same manner. It was found that the factor did not vary between individuals and remained constant from day to day, agreeing closely with the factor found by Kennedy. (See Table VII)

It seems that the blame for discordant results for the absorption factor for chicken hemoglobin must be blamed on the extraordinary heterogeneous character of chicken blood, which made it necessary to base the results on a large number of determinations statistically treated. Results show (Figure II) a higher absorption factor than applies to the blood of mammals with a decided maximum at 12.

The consistent results obtained in analyzing beef blood, which also check with results obtained by Kennedy indicate that it is neither the method which is at fault nor the manipulation. The only logical explanation of these inconsistent results is the peculiar character of the fowl blood itself. When mixed with the Saponin-Potassium Ferricyanide solution in the Van Slyke reaction chamber, it becomes a sticky, viscous ropey mass, and in this form may entangle the oxygen in such a way that it is not all released after three minutes shaking. If this were the case, the concentration would be found to be lower and then a smaller

optical absorption factor would be obtained; this may account for the small factors found, but not the high ones.

Another explanation of these inconsistent results is that the chicken blood contains some other optically absorbent material besides hemoglobin; this would lead to a higher optical density, and thus also to a smaller absorption factor.

Still another possibility is that the hemoglobin of fowl blood has the power of combining with a greater amount of oxygen than has the hemoglobin of mammalian blood. In the latter case the concentration determinations would be high and consequently a higher absorption ratio would be obtained. This would account for the great number of factors above 1.165. At any rate, whatever the cause, a significantly higher optical absorption factor is found for chicken blood based on 100 determinations than applies to beef hemoglobin.

Assuming that the irregularities are caused by the heterogeneous nature of chicken blood, the maximum in the distribution curve rather than the average of all determinations was taken as the final result.

It is obvious from the data here presented that hemoglobin determinations on chicken blood can be very misleading, even when utmost precaution is observed. The spectrophotometric method seems to offer the simplest and most direct accurate means of analysis. But the blood must be diluted at least 1-250 and vigorously shaken to disperse insoluble material.

SUMMARY

1. One hundred chicken blood samples were analyzed for hemoglobin content by the Van Slyke method.

2. The same samples were analyzed with the Bausch and Lomb spectrophotometer. The maxima and minima points of absorption were determined and the absorption factor at the principle maximum found.

3. The absorption factors were plotted against the number of determinations and a maximum obtained at 1.30 mg. hemoglobin per c.c.

4. Nine beef blood samples were analyzed in the same manner and a constant absorption ratio obtained at 1.165 mg. hemoglobin per c.c.

5. Twenty turkey blood samples were analyzed in the same manner as the chicken blood and a similar inconstancy of absorption factors obtained.

It was concluded that:

6. The inconstancy of absorption factors obtained was due to the heterogeneous character of the fowl blood itself and possibly retention of oxygen.

7. Spectrophotometry gave excellent checks on duplicate samples, whereas the oxygen capacity method did not check as consistently on the same sample of blood.

Table II

ABSORPTION SPECTRUM OF [illegible]
([illegible] dilution, depth [illegible] mm.)

Wavelength (mμ)	Optical Density	Wavelength (mμ)	Optical Density
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
579	[illegible]	[illegible]	[illegible]
578	[illegible]	[illegible]	**[illegible]
577	*[illegible]	641	[illegible]
[illegible]	[illegible]	640	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	*[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
		[illegible]	[illegible]

* Maximum

** Minimum

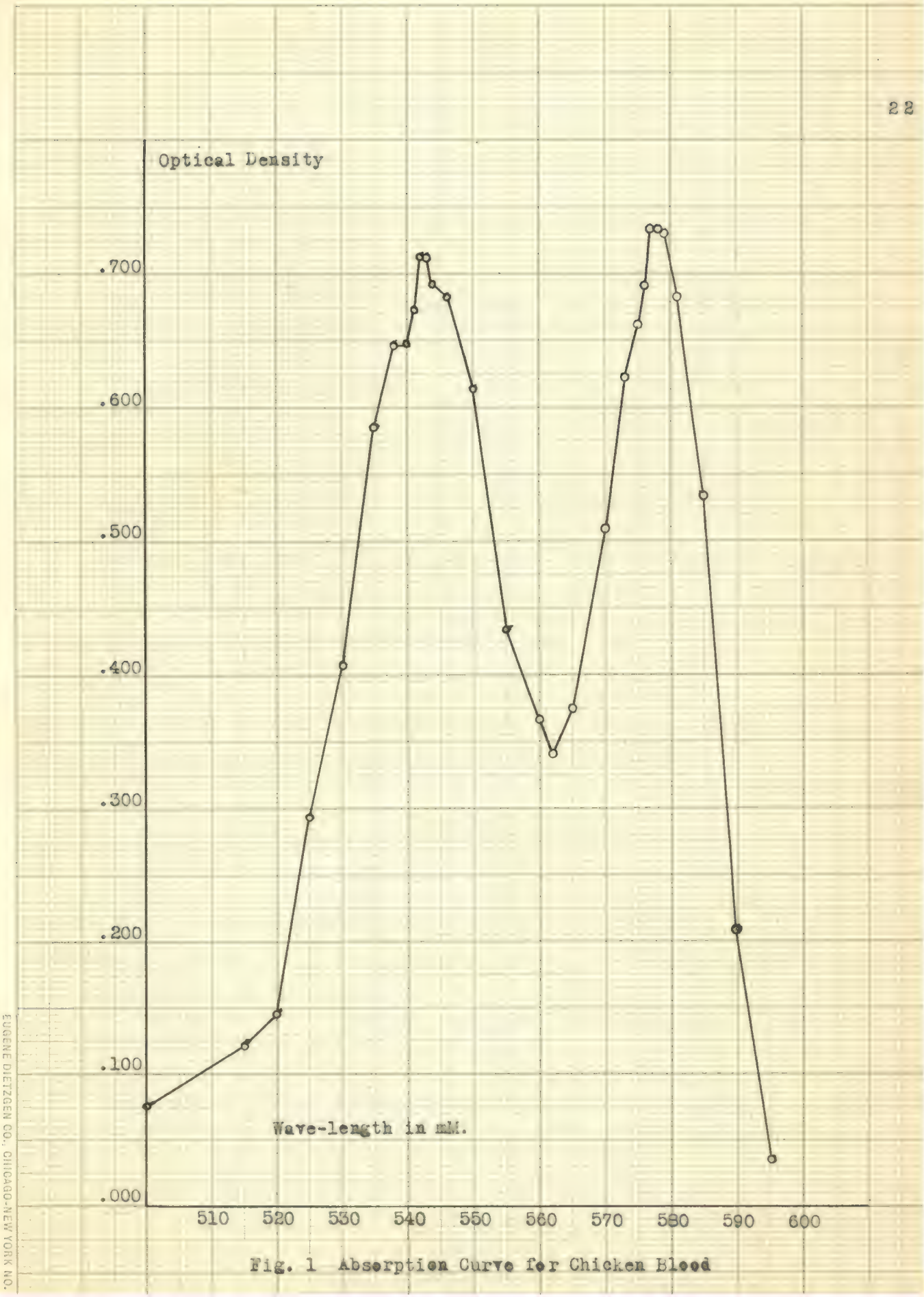

Fig. 1 Absorption Curve for Chicken Blood

Table III

OPTICAL DENSITY RATIOS AT THE CRITICAL POINTS IN THE ABSORPTION SPECTRUM OF CHICKEN HEMOGLOBIN

Animal Number	$\frac{D(542)}{D(562)}$	$\frac{D(577)}{D(562)}$	$\frac{D(577)}{D(542)}$
21	1.61	1.67	1.04
23	1.63	1.63	1.047
21	1.64	1.69	1.06
4	1.68	1.67	1.05
26	1.64	1.68	1.08
9	1.65	1.69	1.04
45	1.60	1.68	1.08
47	1.64	1.70	1.07
1	1.61	1.69	1.08
21	1.61	1.67	1.04
23	1.61	1.70	1.06
4	1.60	1.68	1.05
9	1.65	1.69	1.04
23	1.63	1.69	1.06
26	1.64	1.70	1.03
9	1.63	1.67	1.08
21	1.64	1.68	1.06
4	1.62	1.67	1.05
44	1.64	1.7	1.05

Table III (Con.)

Animal Number	$\frac{D(542)}{D([illegible])}$	$\frac{D(577)}{D(552)}$	$\frac{D(577)}{D(542)}$
46	1.63	1.67	1.03
21	1.65	1.67	1.04
23	1.61	1.67	1.06
26	1.64	1.68	1.08
4	1.63	1.67	1.06
9	1.65	1.68	1.03
Average	1.632	1.68	1.054

Table IV

ABSORPTION SPECTRUM OF BEEF HEMOGLOBIN
(1-100 dilution; depth 1 cm.)

Wavelength (mμ)	Optical Density	Wavelength (mμ)	Optical Density
640	.02235	560	.85024
610	.12950	555	.91398
600	.10338	550	1.1004
595	.25376	546	1.27828
590	.45406	544	1.31615
585	.83650	543	1.33420
581	1.25950	542	*1.36407
579	1.30437	541	1.33428
578	1.39886	540	1.31291
577	*1.41619	538	1.28476
576	1.38084	535	1.10685
575	1.35514	530	.84254
573	1.30194	520	.60302
570	1.1020	510	.51275
565	.86389	500	.58058
562	".84355		

* Maximum

" Minimum

Table V

ABSORPTION FACTORS FOR TURKEY BLOOD
(1-100 dilution, depth 1 cm.)

Animal Number	Partial Pressure of Oxygen	Hb. gr./100 cc. Blood	Optical Density at 542 mμ	Absorption Factor*
1	7.6	12.23	1.172	1.004
2	7.5	12.25	1.152	1.05
3	9.3	14.71	1.198	1.22
4	8.9	14.05	1.412	1.00
5	8.7	14.16	1.37	1.25
6	8.3	14.45	1.44	1.00
7	8.5	13.62	1.44	0.97
8	9.5	15.35	1.48	1.04
9	9.0	14.52	1.51	1.05
10	8.4	13.49	1.39	1.03
11	9.2	15.07	1.42	1.09
12	9.0	14.00	1.40	1.00
13	9.5	15.49	1.31	1.16
14	8.4	13.59	1.27	1.05
15	9.9	14.50	1.35	1.14
16	9.0	14.62	1.41	0.98

*Absorption factor converts optical density to mg. of Hb. per 100 cc. of blood.

Table VI

ABSORPTION FACTORS FOR CHICKEN BLOOD
(1-250 dilution, depth 2.0 cm.)

Animal Number	Partial Pressure of Oxygen	No. gr./100 cc. Blood	Optical Density at 542 mμ	Absorption Factor
27	7.8	12.40	1.25	1.00
1	6.7	10.49	1.05	1.00
2	5.5	8.45	0.83	1.01
27	7.8	12.30	1.30	1.05
19	8.2	13.27	1.38	1.05
3	7.2	11.49	1.09	1.05
27	7.3	11.84	1.11	1.05
2	5.4	8.40	0.788	1.05
25	9.2	14.90	1.38	1.07
25	9.1	14.00	1.37	1.07
26	7.8	12.40	1.17	1.07
26	8.8	13.00	1.21	1.07
1	8.9	14.20	1.32	1.07
25	8.0	12.85	1.16	1.08
17	6.8	10.70	0.97	1.10
25	9.1	15.00	1.17	1.11
17	8.0	12.35	1.15	1.12
26	7.9	12.60	1.15	1.12
21	8.2	14.00	1.25	1.13
21	9.0	14.80	1.29	1.15

Table VI (Con.)

Animal Number	Partial Pressure of Oxygen	Hb. gr./100 cc. Blood	Optical Density at 542 mμ	Absorption Factor
9	8.3	15.40	1.19	1.17
35	8.2	15.60	1.17	1.14
31	8.1	14.76	1.29	1.14
31	7.3	11.70	1.03	1.14
36	8.2	15.27	1.16	1.14
9	8.0	13.00	1.12	1.15
21	8.3	15.95	1.19	1.16
40	6.0	9.32	0.81	1.15
40	6.0	9.32	0.81	1.15
40	6.0	9.32	0.81	1.15
18	6.9	10.70	0.92	1.15
9	8.2	13.30	1.13	1.17
22	7.2	11.48	0.98	1.17
18	6.3	10.70	0.91	1.17
19	6.9	10.90	0.927	1.17
5	7.7	12.35	1.05	1.18
4	9.3	16.00	1.33	1.19
66	9.3	16.30	1.35	1.20
4	9.5	15.50	1.30	1.20
66	9.8	16.20	1.35	1.20
4	9.35	16.10	1.29	1.20
23	8.3	13.27	1.10	1.20

Table VI (Con.)

Animal Number	Partial Pressure of Oxygen	Mb. gr./100 cc. Blood	Optical Density at 542 mμ	Absorption Factor
66	8.8	14.36	1.14	1.20
4	9.0	15.40	1.28	1.20
66	8.6	13.90	1.15	1.20
4	9.1	14.80	1.20	1.20
66	8.8	14.04	1.17	1.20
4	9.0	12.90	1.07	1.20
4	8.0	12.90	1.07	1.20
26	7.5	12.10	1.01	1.20
26	7.4	11.96	0.99	1.20
4	8.4	13.57	1.13	1.20
57	6.6	10.64	0.955	1.20
32	7.1	11.60	0.964	1.20
5	7.5	12.10	1.05	1.20
49	5.8	9.42	0.70	1.20
19	7.8	12.40	1.05	1.20
8	7.8	12.40	1.05	1.20
49	6.0	9.32	0.77	1.20
8	7.6	11.80	0.98	1.20
19	7.9	12.68	1.05	1.20
17	7.5	12.00	1.00	1.20
1	8.2	13.10	1.09	1.20
1	8.8	13.42	1.11	1.20

Table VI (Con.)

Animal Number	Partial Pressure of Oxygen	Hb. gr./100 cc. Blood	Optical Density at 542 mμ	Absorption Factor
19	8.1	12.90	1.07	1.20
1	8.2	13.08	1.08	1.20
5	7.7	12.38	1.05	1.20
5	7.6	11.80	0.97	1.21
2	5.0	7.53	0.62	1.21
52	6.2	9.69	0.80	1.21
57	6.7	10.40	0.85	1.22
39	5.7	8.80	0.70	1.22
52	6.8	10.80	0.88	1.22
9	8.2	13.20	1.08	1.22
1	8.2	13.18	1.06	1.25
25	8.0	12.90	1.05	1.25
25	8.0	12.90	1.04	1.25
9	8.2	13.20	1.08	1.25
25	8.0	12.90	1.05	1.25
9	8.3	13.40	1.07	1.24
66	6.0	9.40	0.74	1.24
9	7.8	12.40	1.00	1.24
4	8.4	14.00	1.12	1.24
9	7.8	12.60	1.02	1.24
9	8.0	13.04	1.04	1.24
2	7.1	11.50	0.92	1.24

Table VI (Con.)

Animal Number	Partial Pressure of Oxygen	Hb. gr./100 cc. Blood	Optical Density at 542 mμ	Absorption Factor
66	6.5	10.21	0.80	1.25
57	6.7	10.40	0.83	1.25
1	8.5	13.90	1.12	1.25
19	7.5	11.65	0.90	1.25
4	5.7	8.90	0.70	1.25
2	6.1	9.58	0.75	1.25
2	7.2	11.60	0.91	1.25
21	8.4	13.57	1.08	1.25
21	8.0	13.00	1.04	1.25
1	8.8	14.00	1.02	1.30
5	8.1	12.90	0.98	1.30

*Absorption factor converts optical density to mg. of Hb. per 100 cc. of blood.

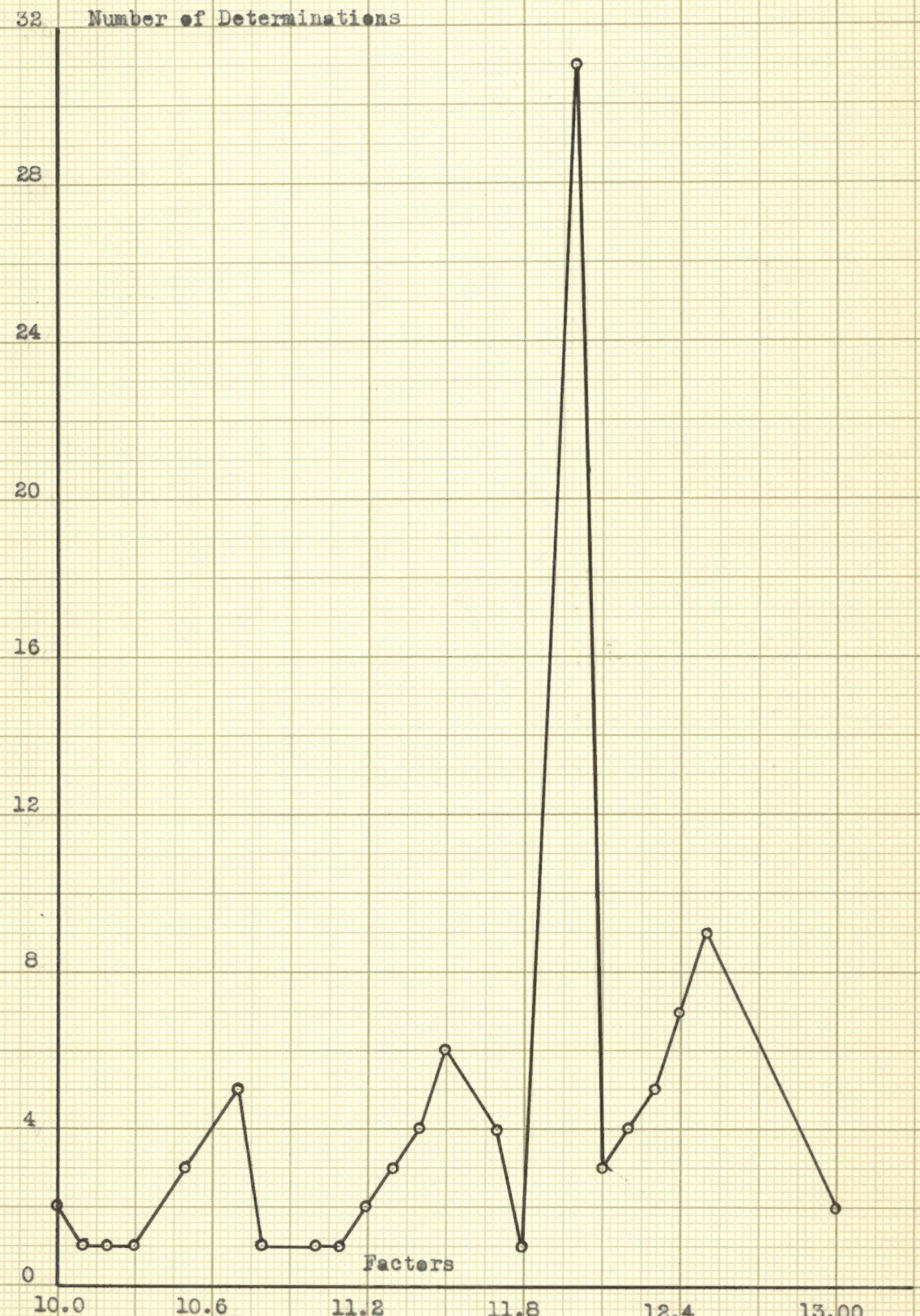

Fig. 2 Absorption Factors, statistically treated.

Table VII

DETERMINATIONS ILLUSTRATING CONSTANCY OF ABSORPTION FACTOR OF HEMOGLOBIN OF CALF BLOOD
(1-100 dilution, depth 1 cm.)

Animal Number	Partial Pressure of Oxygen	Hb. gr./100 cc. Blood	Optical Density at 542 mM	Absorption Factor*
26	6.5	10.30	0.88	1.165
25	7.4	11.90	1.02	1.166
27	7.3	11.78	1.01	1.165
25	7.4	11.90	1.02	1.166
26	6.5	10.25	0.88	1.165
27	7.3	11.78	1.01	1.165
25	7.2	11.48	0.99	1.164
26	6.5	10.30	0.88	1.165
27	7.4	11.90	1.01	1.1648
4	7.3	11.76	1.01	1.165

*Absorption factor converts optical density to mg. of hb. per 100 cc. of blood.

LITERATURE CITED

(1) Twyman, 1930. Journ. Soc. Chem. Ind. 49, 535.

(2) Twyman, 1930. Journ. Soc. Chem. Ind. 49, 667.

(3) Kennedy, R.P. 1926. Am. Journ. Physiology 79, 346.

(4) Fritsch, G.F., 1920. Pfluger Archiv. Fur die Gesammte Physiologie, V 181.

(5) Burker, K., 1913. Zeitschr. f. Biologie Bd. 61, 455.

(6) Van Slyke, D.D., 1918. Journ. Biol. Chem. LXI, 523.

(7) Van Slyke, D.D., 1915. Journ. Biol. Chem. XXXIII, 127.

(8) Scientific Papers of the Bureau of Standards, No. 440, Vol. 18.

(9) Shenk, John H., 1931. Master's Thesis, K.S.C.

*Hari, 1917. Biochem. Zeitschr LXXXII, 229

*Hari, 1919. Biochem. Zeitschr XCV, 257

*Butterfield, 1912. Zeitschr. f. Physiol. Chem. LXXIX.

*Barcroft, 1924. Proc. Roy. Soc. B. XCVII, 61.

*See Table I, p. 9.

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